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# Commonalities and contrasts in recent guidelines for lupus anticoagulant detection

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## SUMMARY

Updates to guidelines covering lupus anticoagulant (LA) detection have recently been published by the International Society on Haemostasis and Thrombosis (ISTH) and British Committee for Standards in Haematology (BCSH), in 2009 and 2012, respectively. The Clinical and Laboratory Standards Institute (CLSI) published its first LA guideline in 2014. Cross-panel agreement exists on sample manipulation, use of dRVVT analysis, conversion of clotting times to ratios, calculations to demonstrate phospholipid dependence, mixing test interpretation and provision of interpretive reporting. Whilst ISTH restricts assay choice to just dRVVT and activated partial thromboplastin time, BCSH and CLSI consider the case for additional tests. All panels acknowledge the potential for false-negative mixing tests, yet they remain mandated by ISTH and BCSH, the latter, however, indicating that a negative mixing test need not exclude a LA if testing on undiluted plasma is unequivocal. CLSI reprioritizes test order to screen-confirm-mix to reduce false-negative reporting when antibodies are diluted to undetectable levels in mixing tests. Recommendations differ on how to derive cut-off levels, particularly in view of the realities of relatively low donor numbers. Each guideline considers testing of anticoagulated patients, BCSH and CLSI endorsing Taipan snake venom time as a useful supplementary assay in patients on vitamin K antagonists. Although full consensus is not apparent, these publications represent significant moves towards engendering common practices.

## INTRODUCTION

Lupus anticoagulants (LA) are somewhat enigmatic in that we detect them largely by inference after exclusion of other causes of our findings. Antibody heterogeneity and reagent variation have thus far prevented generation of reference material or a gold standard assay, which together with analytical platform differences and alternative approaches to raw data manipulation and interpretation, they conspire against


 

our needs and desires for common and ideal diagnostic practices.

Consensus expert guidelines from different committees have been published and updated in recent decades in response to increasing knowledge and experience, the most recent of which are covered herein. An update of the ISTH guideline was published in late 2009 (ISTH 2009) [1] and was followed by an update of the guideline from the British Committee for Standards in Haematology in early 2012 (BCSH 2012) [2]. The first LA detection guideline from the Clinical and Laboratory Standards Institute was published in early 2014 (CLSI 2014) [3]. Whilst incomplete agreement is apparent, consensus guidelines relying as much on personal opinion and experience as objective evidence [4], the commonalities are encouraging in terms of movement towards wider consensus and the contrasts offer informed debate.

## PRE-EXAMINATION

Plasma for LA analysis must be rendered platelet poor, as residual platelet material can shorten clotting times and generate false-negative results. All three guidelines reject use of plasma filtration through 0.22-μm cellulose acetate filters as it introduces variables, not least of which is reduction in sample integrity. Double centrifugation of blood collected into 0.109 M trisodium citrate to achieve a final platelet count of $<10 \times 10^9$/L is advocated by each panel. CLSI 2014 indicates that it is possible to do so with a single centrifugation, although this should be validated as centrifugation type and speed, and operator technique, will affect suitability. Ultracentrifugation in the second step is discouraged by BCSH 2012 and CLSI 2014 due to the potential for microparticle generation.

Each guideline considers that routine coagulation screening is useful prior to performing LA assays to exclude undiagnosed coagulopathies or undisclosed anticoagulant therapy. CLSI 2014 additionally recommends that the routine APTT reagent is LA-unresponsive and any APTT-based testing for LA is performed with a separate, LA-responsive reagent. This reduces chance findings of LA in asymptomatic patients and permits interpretation of LA-responsive assays unencumbered by the need to exclude coexisting abnormalities.

## DETECTION BY INFERENCE

Lupus anticoagulants are detected by employment of the screen-mix-confirm test medley applied to each test type. Screening tests are designed to be LA-responsive, performing the screening test on a mixture of test and normal plasma evidences inhibition, and confirmatory tests are usually a LA-unresponsive version of the screening test and should therefore generate shorter clotting times. The main problem is that of specificity because all LA assays are 'global' tests. Straightforward interpretation of the screen-mix-confirm composite assumes no other causes of elevated clotting times are present, yet this is not always the case. Test principle and reagent design dictate the degree to which different test types are affected by interfering factors. All three documents consider the effects of therapeutic anticoagulation, and CLSI 2014 gives additional detail, as summarized in Table 1.

## WHICH SCREENING TESTS TO USE?

It has been established for some time that antibody and reagent heterogeneity necessitate screening with multiple assays of differing analytical principles to achieve acceptable detection rates [1–3, 5, 6]. ISTH 2009 has introduced a new dimension to this issue by restricting assay choice to only dilute Russell's viper venom time (dRVVT) and activated partial thromboplastin time (APTT) with low phospholipid concentration, stating that the risk of false-positive results is increased to an unacceptable level if more tests are performed. This assertion is further supported by statements concerning analytical difficulties ascribed to other test methods. Although no evidence is cited for increasing false positivity as more tests are performed, it is nonetheless a theoretical possibility. Whether a cut-off has been derived from 95th, 97.5th or 99th percentile, a proportion of normal individuals will be natural statistical outliers and the likelihood of a false-positive screening test from any individual may well increase with test numbers performed.

Clinical and Laboratory Standards Institute 2014 counters this premise by indicating that although false-positive screening tests are inevitable, subsequent performance of the confirmatory and mixing tests will not normally lead to a false-positive interpretation of the composite. Outliers and non-LA abnormalities should generate confirm results similar

**Table 1.** Interfering factors in lupus anticoagulant assays

| Assays grouped by type | Non-LA causes of elevated clotting times for each assay type group |
|---|---|
| Intrinsic pathway-based assays* | |
| APTT, Dilute APTT, KCT, SCT | Deficiencies of factors I, II, V, VIII, IX, X, XI, XII, prekallikrein & HMWK<br>Anticoagulation with VKA, UFH, LMWH, DXa, DTI<br>Nonphospholipid-dependent inhibitors (i.e. factor specific) |
| Extrinsic pathway-based assays | |
| dPT, ASLA | Deficiencies of factors I, II, V, VII, X (FVIII & IX may affect dPT)<br>Anticoagulation with VKA, DXa, DTI (UFH if no neutralizer)<br>Nonphospholipid-dependent inhibitors (i.e. factor specific) |
| Common pathway-based assays (FX activation) | |
| dRVVT, VLVT | Deficiencies of factors I, II, V, X<br>Anticoagulation with VKA, DXa, DTI (UFH if neutralizer quenched)<br>Nonphospholipid-dependent inhibitors (i.e. factor specific) |
| Common pathway-based assays (FII activation) | |
| TSVT, Textarin time | Deficiencies of factors I, II (FV for Textarin time)<br>Anticoagulation with DTI, UFH<br>Nonphospholipid-dependent inhibitors (i.e. factor specific) |

APTT, activated partial thromboplastin time; KCT, kaolin clotting time; SCT, silica clotting time, dPT, dilute prothrombin time; ASLA, activated seven lupus anticoagulant assay; dRVVT, dilute Russell's viper venom time; VLVT, Vipera lebetina clotting time; TSVT, Taipan snake venom time; HMWK, high molecular weight kininogen; VKA, vitamin K antagonist; DXa, direct FXa inhibitor; DTI, direct thrombin inhibitor; UFH, unfractionated heparin.
*Elevated FVIII and/or fibrinogen can shorten clotting times and mask LA.

to screen results, and genuine LA unreactive in other reagents [7] will not go undetected.

There is a considerable body of evidence indicating that dRVVT and APTT are an effective pairing for LA detection, so the ISTH 2009 recommendation is not without firm foundation and additionally serves to promote common diagnostic practices. BCSH 2012 and CLSI 2014 concur that dRVVT should be one of the tests performed, which is entirely unsurprising as it is known to be sensitive to β2glycoprotein I-dependent antibodies and highly correlate with thrombosis [8]. BCSH 2012 recommends employing two tests, suggested potential pairings for dRVVT being APTT with proven LA sensitivity, modified APTT or dilute prothrombin time (dPT). CLSI 2014 recommends LA-responsive APTT as a minimum partner for first-line screening but does not exclude use of other assays at initial testing or as second-line assays. It is, however, important to be cognisant of variability in diagnostic performance for dRVVT and APTT reagents from different manufacturers [1, 3, 6, 9, 10].

Interestingly, only ISTH 2009 specifically recommends use of dilute phospholipid in APTT, whilst recommendations in the other two guidelines can include routine APTT reagents considered LA-responsive despite 'standard' phospholipid concentration. Both approaches have limitations. A LA-unresponsive phospholipid preparation will not necessarily perform better upon dilution [5], yet an undiluted LA-responsive preparation may be sufficiently concentrated to overcome weaker antibodies [8].

### Do other tests have a role in LA detection?

British Committee for Standards in Haematology 2012 and CLSI 2014 promote tests other than dRVVT and APTT based on not inconsiderable experience of their diagnostic application and recognition that these assays can detect clinically significant antibodies unreactive in dRVVT or APTT, as well as most that are [7].

Kaolin clotting time (KCT) is not recommended in ISTH 2009 as it is said to be less reproducible than other tests. This is indeed the case and arises from issues such as long clotting times, residual plasma lipid variation and necessity for meticulous plasma preparation. CLSI 2014 evidences that KCT can be a sensitive APTT-based assay in experienced hands and does not discount its use. An important limitation of KCT not receiving attention in any document is the lack of a widely employed confirmatory test, which compro-

mises specificity by not evidencing phospholipid dependence. A further limitation of KCT quoted in ISTH 2009 is the incompatibility of kaolin with analysers employing photo-optical clot detection, although low-opacity/slow-settling KCT reagents are in fact commercially available. An initial response to this issue was description of a modification of KCT with a photo-optical compatible contact activator, the silica clotting time (SCT), yet the reproducibility issues remained. It has been further modified into a paired system with dilute and concentrated phospholipid in the screen and confirm tests, respectively, ostensibly converting it into an automate-friendly dilute APTT and has shown good diagnostic performance in partnership with dRVVT [11].

International Society on Haemostasis and Thrombosis 2009 does not recommend ellagic acid as the APTT contact activator, indicating that it is insensitive to LA. Kumano *et al.* [12] have recently shown that ellagic acid-containing reagents can be suitable for LA detection as it is the phospholipid that is responsible for between-reagent differences in LA responsiveness. Neither BCSH 2012 nor CLSI 2014 restricts choice of APTT reagent based on the contact activator, although it should be acknowledged that some commercially available ellagic acid-containing APTTs have been shown to have lower sensitivity, albeit coincidental to their phospholipid composition [12, 13].

Thromboplastin variability is given as the reason that dilute prothrombin time (dPT) is no longer endorsed by ISTH. Although it has been established that recombinant thromboplastins have greater LA-sensitivity and less variability than tissue-derived reagents [3, 7, 8], a compounding problem has been that of numerous local technique modifications [10]. BCSH 2012 and CLSI 2014 consider that there is sufficient evidence and experience for dPT that it should not be discounted as a member of a screening panel, especially considering it has been shown to detect clinically significant antibodies, some unreactive with dRVVT and APTT [7, 14, 15]. Evaluations of a standardized commercially available reagent kit have given rise to the suggestion that addition of dPT to an APTT and dRVVT repertoire will increase detection rates [7, 14].

Assays based on snake venoms other than Russell's viper venom are not recommended in ISTH 2009 as it claims that standardized commercial assays are unavailable. Although this is the case for Textarin, reagents for LA detection by Taipan snake venom time (TSVT) screening and ecarin time (ET) for confirmation are commercially available [7, 16]. CLSI 2014 briefly mentions a recently described alternative to dRVVT, the *Vipera lebetina* venom time (VLVT), which employs the FX activator from Blunt-nosed viper venom. There are limited published data evidencing equivalence between dRVVT and VLVT, and they cannot yet be considered interchangeable.

It is important to consider that new or alternative assays tend to be evaluated against at least dRVVT, and commonly in conjunction with APTT. This creates a selection bias as other assays can be sensitive to subpopulations of clinically significant antibodies [7, 14–18]. Nonetheless, a well-performed dRVVT/APTT pairing has repeatedly been shown to be sensitive to most LA, and whilst there is a case for alternative assays in certain circumstances [2, 3, 7, 14–18], adoption of wider repertoires is probably unnecessary for all but reference laboratories or those with specific interest.

## RATIOS

Previous recommendations to convert screen and confirm clotting times to ratios via normal pooled plasma (NPP) values are retained in BCSH 2012 and adopted in ISTH 2009. This practice reduces the impact of variables such as operator and/or analyser performance, reagent quality and stability issues, and variation in NPP clotting time with different reagents, which can occur even with paired screen and confirm reagents. CLSI 2014 instead recommends normalization against the reference interval (RI) mean clotting time as not all NPPs, or batches of the same NPP, generate the same clotting times with different reagents of the same test type [9, 19]. A NPP value towards or beyond an extreme of a RI can lead to false-positive or false-negative results [19], as shown in Table 2. Although an immediate response to this might be to select a NPP with values close to the RI mean, you can see from Table 2 that this could necessitate employing a different NPP for each test. This would be cumbersome and expensive in terms of identifying suitable NPPs, and more so, whenever a new batch of a given NPP performs differently.

Aside from negating NPP variability, the rationale for this recommendation parallels standard practice in any coagulation laboratory generating international

**Table 2.** Impact of unsuitable normal pooled plasmas on lupus anticoagulant assay interpretation

| Assay | dRVVT screen | dRVVT confirm | dAPTT screen | dAPTT confirm |
|---|---|---|---|---|
| RI for clotting time (s) | 35.9–51.7 | 30.8–42.3 | 34.7–48.2 | 39.0–54.0 |
| RI for ratio | 0.82–1.18 | 0.82–1.13 | 0.84–1.16 | 0.85–1.18 |
| RI mean (s) | 43.8 | 37.6 | 41.4 | 45.9 |
| Mean clotting time for commercial lyophilized NPP (s) | 47.4 | 35.9 | 42.8 | 46.8 |
| Mean clotting time for commercial frozen NPP (s) | 44.0 | 37.8 | 36.0 | 42.8 |
| Mean clotting time for locally prepared frozen NPP (s) | 44.8 | 34.8 | 38.1 | 40.3 |
| Application of NPP *vs.* RI mean as denominator | | | | |
| False-negative dRVVT screen | dRVVT | 54.7s | Ratio of 1.15 via commercial lyophilized plasma<br>Ratio of 1.25 via RI mean | |
| False-positive dAPTT screen | dAPTT | 46.0s | Ratio of 1.28 via commercial frozen plasma<br>Ratio of 1.11 via RI mean | |
| False-negative dAPTT interpretation | dAPTT | 51.6s | Ratio of 1.35 via locally prepared frozen plasma<br>Ratio of 1.25 via RI mean | |
| | dAPTT confirm | 50.5s | Ratio of 1.25 via locally prepared frozen plasma<br>Ratio of 1.10 via RI mean | |
| | % correction | 7.4<br>12.0 | Ratios via locally prepared frozen plasma<br>Ratios via RI mean | |

Theoretical raw clotting times applied to reference intervals and mean clotting times taken from [19] to demonstrate potential of NPPs with clotting times distant from RI means to generate inaccurate interpretation.
RI, reference interval; NPP, normal pooled plasma; dRVVT, dilute Russell's viper venom time; dAPTT, dilute activated partial thromboplastin;% correction, per cent correction of screen ratio by confirm ratio (cut-off: 10%) [2, 6, 9].

normalized ratios (INRs) via geometric mean normal prothrombin times. CLSI 2014 additionally refers the reader to its own guideline for establishing RIs regarding generation of data over a number of days to account for overall technique variation.

## GENERATING REFERENCE INTERVALS/ CUT-OFF LEVELS

The first step in LA result interpretation is determining whether any screening test result is above the upper limit of locally derived RIs specific to the reagent-analyser pairings [1–3, 6, 9, 20]. If so, mixing and confirmatory tests can be initiated. BCSH 2012 indicates that RIs for LA assays have historically been derived from the RI mean ± 2 standard deviations (SD) from normal donor populations. A Gaussian distribution, common in 'global' clotting assays [3], thus generates a cut-off at the 97.5th percentile, necessitating recognition that approximately 2.5% of normal patients will be outliers with results above this value. ISTH 2009 recommends cut-offs derived from the 99th percentile, equating to the RI mean + 2.3 SD for normally distributed data. This introduces potential to increase specificity by reducing the frequency of false-positive screening tests, although BCSH 2012 clarifies the statistical inevitability of a consequent reduction in sensitivity.

The ISTH 2009 recommendation has proven further contentious as it indicates that a 99th percentile value can be derived from a 40 donor minimum. A minimum of 120 donors has been previously recommended for normally distributed data to generate an accurate 99th percentile and significantly more for non-normally distributed data [13, 21]. BCSH 2012 gives attention to the under-appreciated issue of inaccuracy of RI genera-

tion with small sample sizes irrespective of distribution or preferred percentile and cites the option of validating previously established cut-offs from smaller numbers (20–60) of normal donors [21]. CLSI 2014 quotes the same source (a separate CLSI publication), to maintain that generating a RI from its mean ± 2 SD is a valid diagnostic tool and echoes the BCSH 2012 advocation of validating established cut-offs with reduced donor numbers. The CLSI 2014 contention about not generating false-positive interpretations when employing additional screening tests holds true here too – a false-positive screening test from a statistical outlier of the 97.5th percentile will not generate false-positive composite interpretation, yet a false-negative resulting from the reduced sensitivity inherent with application of the 99th percentile could lead to failure to secure appropriate diagnosis and treatment.

## MIXING TESTS

Lupus anticoagulants are by definition inhibitors and performance of the screening test on a mixture of test plasma and NPP to evidence this property is a mainstay in LA detection. All three guidelines concur that this should be performed on a 1 : 1 mixture, and the NPP must be appropriately prepared to be platelet poor. ISTH 2009 is the first guideline to proffer definitive guidance on mixing test interpretation, indicating that either a mixing test-specific cut-off [22] or application of the Index of Circulating Anticoagulant calculation [23] are equally valid options. CLSI 2014 makes identical recommendations but the issue is not covered in BCSH 2012.

Recently, the reliability of mixing tests in LA detection has been questioned, with particular reference to the propensity for LA to be diluted to apparently undetectable levels [5–8, 16, 22, 24, 25]. This can be a problem when, as indicated in ISTH 2009, the immediate response to an elevated screen is to perform the mixing test and use that as a decision point for whether to perform the confirm. BCSH 2012 states that mixing tests improve specificity, but introduce a dilution factor that may make weak antibodies appear negative. In terms of practical guidance, it states that in the absence of other causes of prolonged clotting times, samples with negative mixing tests but positive screen and confirm tests on undiluted plasma can be considered LA-positive.

Clinical and Laboratory Standards Institute 2014 takes this paradigm a step further and reprioritizes the order of testing to screen then confirm, and only performing the mixing test if initial testing is not clear cut. Otherwise, analytical limitations of mixing tests can lead to false-negative reporting [5–8, 16, 22, 24, 25]. Specific, concurrent criteria are given for when to omit the mixing test:

- Elevated LA-screening test.
- Confirmatory test mathematically corrects screening test and returns into the RI, to exclude a coexistent abnormality.
- No evidence of other causes of elevated clotting times (i.e. from a coagulation screen that includes LA-unresponsive APTT).

## CONFIRMATORY TESTS

There is full agreement that confirmatory tests for demonstrating phospholipid dependence must be based on the screening test(s) that was abnormal. Some laboratories only undertake the mixing test when confronted with an elevated APTT, but not performing APTT-based confirm tests risks loss of specificity and increased false-negative interpretations unless dRVVT testing is also positive.

British Committee for Standards in Haematology 2012 suggests employing high-phospholipid concentration, platelet neutralization procedure (PNP) or LA-insensitive reagent in confirm tests, whilst ISTH 2009 stipulates increasing the phospholipid concentration with bilayer or hexagonal (II) phase phospholipid. CLSI 2014 ostensibly concurs by detailing available confirmatory tests for each screening test, including considerations of limitations.

Another welcome first for ISTH is a recommended calculation for evidencing phospholipid dependence, the percentage correction of screen by confirm. This has been endorsed in BCSH guidelines since 1991 and is given by CLSI 2014 as an option for dRVVT. CLSI 2014 offers alternatives specific to particular assays, such as deltas for hexagonal phase phospholipid neutralization test and PNP as commonly used for APTT, whilst the normalized screen to confirmatory ratio (screen ratio/confirm ratio) is recommended for paired dRVVT, SCT and dPT screen and confirm reagents. BCSH 2012 gives the latter as an alternative to per cent correction of ratio.

## INTEGRATED TESTING

Integrated testing involves performing screen and confirm (i.e. low and high phospholipid) simultaneously on every patient and directly calculating per cent correction or screen/confirm ratio, irrespective of whether the screen alone is elevated. A particular advantage of this approach is with weaker LA, where the patient's basal clotting time is prolonged but not to a sufficient extent that it exceeds the cut-off, yet the screen and confirm discrepancy reveals the antibody [26, 27]. CLSI 2014 suggests that 'true' integrated tests are those that also incorporate dilution in NPP, whilst BCSH 2012 does not cover the specifics.

### Does integrated testing render mixing tests redundant?

International Society on Haemostasis and Thrombosis 2009 states that integrated testing on undiluted plasma does not, in principle, require subsequent performance of a mixing test. The premise is that the traditional testing order of screen-mix-confirm is circumvented and LA can be detected without performing the mixing test. This has raised concern that ISTH no longer mandates mixing tests, at least in this scenario [28, 29], leading to clarification that this was not the intended message [30].

Some LAs exhibit a degree of resistance against the 'swamping' effect of high-phospholipid reagents and generate results above the confirmatory test RI, which will often but not always be accompanied by a sufficiently higher screen result to nonetheless achieve a positive interpretation [1, 3, 13, 28]. In either instance, there exists the possibility of a concomitant or alternative abnormality. Where a potent LA generates concordantly elevated screen and confirm, the integrated approach will deliver a false-negative interpretation and warrant further investigation [28]. Performing screen and confirm on 1 : 1 mixtures with NPP can be informative in unravelling the cause(s) of such data, as shown in Table 3.

Test and reagent types with higher specificity are more likely to clearly demonstrate the presence of a LA in undiluted plasma and not require mixing tests [31]. The debate is crystallized in the CLSI 2014 recommendations, where it is not a case of performing mixing tests on everyone or no-one, rather the decision is made on a case-by-case-basis.

## TESTING ANTICOAGULATED PATIENTS

It is stated in each guideline that testing for LA in patients receiving vitamin K antagonists (VKA) and therapeutic doses of unfractionated heparin (UFH) are best postponed until a suitable period after discontinuation of anticoagulation. Despite this, diagnostic laboratories continue to receive requests for testing in these circumstances and guidance is required.

### Vitamin K antagonists

International Society on Haemostasis and Thrombosis 2009 indicates that LA assays can be undertaken on undiluted plasma if the INR is <1.5 and on 1 : 1 mixtures with NPP if the INR is between 1.5 and 3.0, with acknowledgement of the dilution effect. Integrated tests and assays based on snake venoms unaffected by the VKA anticoagulant effect are not recommended as it is suggested they require further critical evaluation.

British Committee for Standards in Haematology 2012 and CLSI 2014 specify performance of screen and confirm tests on 1 : 1 mixtures with NPP and place no restrictions based on INR values, as informed data interpretation can reveal LA in some patients with INRs >3.0 [16]. Assays on undiluted plasma may lead to false-positive or false-negative results and are not recommended [6, 9, 13, 16], whilst an elevated screen on the mixture can be taken as grounds to suspect an inhibitor and the confirm will demonstrate phospholipid dependence. Both documents make the crucial point that negative results in mixing studies in such cases do not exclude the presence of a LA because of the dilution effect. TSVT with ET or PNP as confirmatory tests is indicated in both guidelines as useful secondary testing for patients on VKA.

### Heparins

Caution is urged in ISTH 2009 and CLSI 2014 when interpreting results on patients receiving UFH, whilst BCSH 2012 specifies that LA testing should not be performed on patients receiving therapeutic doses as it

**Table 3.** Use of mixing studies when integrated testing is not clear cut

| Integrated testing on undiluted plasma<br>dRVVT screen ratio<br>(RI 0.82–1.18) | dRVVT confirm ratio<br>(RI 0.82–1.13) | % correction<br>(Cut-off: <10%) | Screen/confirm ratio<br>(Cut-off: <1.15) | Interpretation |
|---|---|---|---|---|
| 1.55 | 1.22 | 21.3 | 1.27 | Positive for LA but possible coexisting abnormality |
| 1.55 | 1.51 | 2.6 | 1.03 | Negative for LA |
| **Potential 1 : 1 mixing study results**<br>**dRVVT screen ratio**<br>**(RI 0.90–1.10)** | **dRVVT confirm ratio**<br>**(RI 0.89–1.10)** | **% correction**<br>**(Cut-off: <10%)** | **Screen/confirm ratio**<br>**(Cut-off: <1.15)** | **Interpretation** |
| 1.38 | 1.36 | 1.5 | 1.01 | Nonphospholipid-dependent inhibitor*<br>LA requiring further dilution* |
| 1.01 | 1.02 | −0.99 | 0.99 | Factor deficiency† |
| 1.35 | 1.08 | 20.0 | 1.25 | LA proven‡ |
| 1.92 | 1.09 | 43.2 | 1.76 | LA with co-factor effect |

dRVVT, dilute Russell's viper venom time; RI, reference interval; LA, lupus anticoagulant; % correction, per cent correction of screen ratio by confirm ratio.
*Unlikely mixing test outcomes from undiluted testing with positive interpretation since screen/confirm discordance should remain.
†If undiluted testing gives positive interpretation, these results most likely represent dilution of the LA rather than a factor deficiency being the sole cause of the undiluted plasma results. If these mixing tests accompany a negative interpretation from undiluted plasma, the most likely explanation is factor deficiency and no LA.
‡Factor assays may be indicated, particularly if routine coagulation screen abnormal.

may cause erroneous results. Reagent-integral heparin neutralizers, common in commercial dRVVT reagents, and heparin-neutralizing compounds added to plasma prior to analysis are discussed in CLSI 2014. Heparin neutralizers are effective only up to a specified level, commonly 0.8–1.0 U/mL, and this must be taken into account during result interpretation. A confirmatory test returning into the reference range is a good indicator that heparin has been quenched. LA assays are much less affected by low molecular weight heparins, and, in any case, heparin neutralizers are usually capable of covering prophylactic doses.

### New anticoagulants

Only CLSI 2014 also covers potential interferences by the newer anticoagulants. Direct thrombin inhibitors (DTI) interfere with all LA assays and introduce significant potential for false-positive results. Unlike the situation with VKA, mixing tests will not necessarily correct the effects of DTI or direct factor Xa (FXa) inhibitors as there is usually no underlying factor deficiency [13]. Rivaroxaban interferes with dRVVT more so than APTT and has a variable effect on prothrombin time-based assays. A recent study showed TSVT coupled with ET to be a sensitive pairing for detecting LA, and unaffected by rivaroxaban therapy, as both venoms are prothrombin activators and thus unaffected by FXa inhibition [32].

## REPORTING

All three guidelines state that reports should include an interpretation indicating whether LA has been detected or not. Each supports the recommendation to retest within a minimum of 12 weeks to evidence antibody persistence.

## CONCLUSION

Firm consensus between the panels for issues such as sample manipulation, use of dRVVT, use of ratios,

calculations for phospholipid dependence and inhibition, and interpretive reporting, will do much to engender common practice. There is sufficient evidence and experience for tests other than dRVVT and APTT for two of three expert panels to consider there is a place for them in certain situations. The mixing test debate rages on, although it does seem they can be omitted in specific circumstances and that informed data interpretation is key. Our understanding of the antibodies and assays continues apace, and it may be that the wait and anticipation for further guideline updates will be short.

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